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L2: Entry 1 of 19 File: USPT Aug 7, 2001

DOCUMENT-IDENTIFIER: US 6271002 B1
TITLE: RNA amplification method

BSPR:
Generally, the level of expression of the protein product of a gene and its messenger RNA (mRNA) transcript are correlated, so that measuring one provides you with reliable information about the other. Since in most instances it is technically easier to measure RNA than to measure protein, variations in mRNA levels are commonly employed to assess gene expression in different cells and tissues or in the same cells and tissues at different stages of disease or development or exposed to different stimuli. One particularly useful method of assaying gene expression at the level of transcription employs DNA microarrays (Ramsay, Nature Biotechnol. 16: 40-44, 1998; Marshall and Hodgson, Nature Biotechnol. 16: 27-31, 1998; Lashkari et al., Proc. Natl. Acad. Sci. (USA) 94: 130-157, 1997; DeRisi et al., Science 278: 680-6, 1997).

DEPR:
This invention is particularly useful for the analysis of gene expression profiles. For expression profiling, DNA microarrays are typically probed using mRNA, extracted and amplified from the cells whose gene expression profile it is desired to analyze, using the 3'-end PCR/IVT amplification method of the invention. To facilitate comparison between any two samples of interest, the mRNAs are typically labeled separately with fluorescent dyes that emit at different wavelengths, as described in Section 5.2. Some embodiments of this invention are based on measuring the transcriptional rate of genes.

DEPR:
As noted above, the "binding site" to which a particular cognate cRNA specifically hybridizes is usually a nucleic acid or nucleic acid analogue attached at that binding site. In one embodiment, the binding sites of the microarray are DNA polynucleotides corresponding to at least a portion of each gene in an organism's genome. These DNAs can be obtained by, e.g., polymerase chain reaction (PCR) amplification of gene segments from genomic DNA, cDNA (e.g., by RT-PCR), or cloned sequences. PCR primers are chosen, based on the known sequence of the genes or cDNA, that result in amplification of unique fragments (i.e., fragments that do not share more than bases of contiguous identical sequence with any other fragment on the microarray). Computer programs are useful in the design of primers with the required specificity and optimal amplification properties. See, e.g., Oligo version 5.0 (National Biosciences). In the case of binding sites corresponding to very long genes, it will sometimes be desirable to amplify segments near the 3' end of the gene so that when oligo-dT primed cDNA probes are hybridized to the microarray, less-than-full length probes will bind efficiently. Typically each gene fragment on the microarray will be between about 50 bp and about 2000 bp, more typically between about 100 bp and about 1000 bp, and usually between about 300 bp and about 800 bp in length.

DEPR:
Optimal hybridization conditions will depend on the length (e.g., oligomer versus polynucleotide greater than 200 bases) and type (e.g., RNA, DNA, PNA) of labeled probe and immobilized polynucleotide or oligonucleotide. General parameters for specific (i.e., stringent) hybridization conditions for nucleic acids are described in Sambrook et al. (1989, Molecular Cloning--A Laboratory

Manual (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) and in Ausubel et al. (1987, Current Protocols in Molecular Biology, Greene Publishing, Media, Pa., and Wiley-Interscience, New York). When the cDNA microarrays of Schena et al. (1996, Proc. Natl. Acad. Sci. USA, 93:10614-19) are used, typical hybridization conditions are hybridization in 533 SSC plus 0.2% SDS at 65.degree. C. for 4 hours followed by washes at 25.degree. C. in low stringency wash buffer (1.times.SSC plus 0.2% SDS) followed by 10 minutes at 25.degree. C. in high stringency wash buffer (0.1.times.SSC plus 0.2% SDS) (Schena et al., 1996, Proc. Natl. Acad. Sci. USA, 93:10614-19). Useful hybridization conditions are also provided in, e.g.,Tijssen, 1993, Hybridization With Nucleic Acid Probes, Elsevier Science Publishers B.V., Amsterdam and New York, and Kricka, 1992, Nonisotopic DNA Probe Techniques, Academic Press, San Diego, Calif.

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L2: Entry 5 of 19 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251601 B1
TITLE: Simultaneous measurement of gene expression and genomic abnormalities using nucleic acid microarrays

DEPR:
Exemplary of the types of microarrays useful in the method of the invention is a prenatal array of about 100 target elements without replicates, which comprise genomic DNA sequences from (a) the unique sequence regions immediately adjacent the repeat sequence regions of (i) all human telomeres and (ii) all human centromeres (taken from both p and q arm); (b) the "microdeletion" syndrome regions for DiGeorge, Smith-Magenis, Downs, Williams, Velocardiofacial, Alagille, Miller-Dieker, Wolf-Hirschhorn, Cri du Chat, Cat Eye, Langer-Giedion, Kallmann and Prader-Willi/Angelman syndromes; and (c) deletion regions identified with sterylsulfatase deficiency, muscular dystrophy and male infertility, and those believed tied to mental retardation that involve deletion of the sub-telomeric, unique sequence regions on each chromosome.

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